

Detection (CGH)

Section of Cancer Genomics, Genetics Branch, NCI
National Institutes of Health

Reagents

Antibodies:

Avidin-FITC

Vector, Cat. A-2011

Biotinylated anti-avidin

Vector, Cat. BA0300

Goat anti rabbit TRITC

Sigma, Cat. T-5268

Mouse anti-DIG

Sigma, Cat. D-8156

Rabbit anti mouse TRITC

Sigma, Cat. T-2402

Antifade (1,4-phenylene-diamine)

Bovine Serum Albumin (BSA)

DAPI

Formamide

HCl, 1 M

20X SSC

Tween 20

Water, sterile

Preparation

FA/SSC

20X SSC 30 ml

dH₂O 120 ml

Formamide 150 ml

Adjust pH to 7-7.5 with 1M HCl

(200 ml of FA/SSC requires 500 µl of 1M HCl)

Prewarm to 45°C

4X SSC/Tween 20

20X SSC 100 ml

dH₂O 400 ml

Tween 20 0.5 ml

Prewarm to 45°C

0.1X SSC

20X SSC	2.5 ml
Add H ₂ O to	500 ml

Prewarm to 60°C

Note: For the above wash solutions you need 70 ml for glass coplin jars and 50 ml for plastic jars per step.

Blocking Solution (3% BSA)

Bovine serum albumin (powder)	0.3 g
Pre-warmed (37°C) 4X SSC/Tween 20	10 ml

Vortex until dissolved at 37°C

DAPI (80 ng/ml DAPI in 2X SCC)

Stock solution:

DAPI	2 mg
Sterile water	10 ml

Working solution:

Stock solution	40 µl
2X SSC	100 ml

Antibodies

Layer 1 avidin-FITC (1:200) + mouse-anti-DIG (1:200)

Layer 2 biotinylated anti-avidin (1:200) + rabbit anti mouse TRITC (1:200)

Layer 3 avidin-FITC (1:200) + goat anti rabbit TRITC (1:200)

NOTE: MAKE SURE SOLUTIONS ARE AT THE CORRECT TEMPERATURES BEFORE USING: CHECK WITH A THERMOMETER.

Procedure

1. Remove rubber cement and coverslips from hybridized slides.
2. Wash slides in FA/SSC (use coplin jars), 3 x 5 min, shaking; first dip the slides into FA/SSC to remove coverslips.
3. Wash slides in 0.1X SSC, 3 x 5 min, shaking.
4. Dip slides in 4X SSC/Tween 20; do not let them dry.

5. Add 100 μ l of Blocking Solution to 24 x 60 mm coverslips, touch slides to coverslips, and incubate in hybridization chamber at 37°C for about 30 min.
6. Dip slides in 4X SSC/Tween 20; do not let them dry.

Note: Spin all fluorescent dyes for 3 min at 13,000 rpm.

7. Antibodies: Add 100 μ l of prepared antibody solution (antibodies should be diluted in 1% BSA) to coverslip (use 24 X 60 mm), touch slide to coverslip, and incubate in hybridization chamber for 45-60 min at 37°C.
8. Wash slides in 4X SSC/Tween 20, 3 x 5 min, shaking.
9. Apply second and third antibody layers by repeating steps 7 and 8.
10. DAPI staining: stain for 5 min in foil-covered coplin jar.
11. Wash in 2X SSC for 10 min, shaking.
12. Apply 30-35 μ l antifade, cover with 24 X 60 mm coverslip, and store in the dark at 4°C.